The interplay between genetic and learned components of behavioural traits: olfactory responses of predatory mites to signals contained in a herbivore-induced plant volatile

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This thesis deals with the evolution of behavioural traits that have a genetic basis, yet can be modified by learning in response to environmental influences. It is the tenet of Darwinian theory that natural selection will drive evolution of a trait if there is heritable and selectable variation for a phenotypic trait. Variation is selectable when phenotypes differ in survival chances and reproductive success, i.e., individuals that vary in the trait differ in terms of the number of their offspring. Variation is heritable when each phenotype has, at least partially, a genetic basis. Thus, phenotypic variation arises from differences in the genetic make-up of different phenotypes. Together with recombination, mutation is regarded as the process creating variants of the trait, whereas natural selection is the process eroding genetic variation.

The presence of selectable variation for a phenotypic trait determines its evolutionary potential to respond to natural selection (Houle 1992; Falconer and Mackay 1996). However, selectable phenotypic variation may arise from differences in the genetic make-up of different phenotypes as well as from the way these phenotypes respond to environmental influences (i.e., phenotypic plasticity). If the ability to adaptively respond to environmental influences by producing certain phenotypes has a genetic basis (and is therefore heritable) then this ability can be shaped by natural selection and evolve as well.

Therefore, there is an evolutionary interplay between the innate (i.e., genetically determined) phenotype and the ability to modify it in response to environmental influences. In particular, phenotypic plasticity may change fitness of individuals, and thus it may influence how natural selection acts on the selectable phenotypic variation for the innate trait. At the same time, both the innate phenotype and its plasticity may have genetic bases. Hence, both these two traits may be shaped by natural selection and thus jointly evolve. Thus, evolutionary pathways are possible where the trait evolves primarily via the innate component, or primarily via improved plasticity or a mixture of both (as illustrated by Papaj 1994). Moreover, phenotypic plasticity may play a role in creating novel selectable forms that are entirely environmentally induced when there is not any
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genetic basis for such a variant as, e.g., in populations that colonize a novel environment (ten Cate 2000; Price et al. 2003; West-Eberhard 2005; Crispo 2008).

Learning can be considered as a special form of phenotypic plasticity of behavioural traits because its effect may be reversible; a learned behavioral response may wane if the environmental stimulus that triggered it is no longer present, or it may be modified if a new environmental stimulus occurs. Some mechanisms of learning result in an adaptive change of behaviour, i.e., a change that allows the modified phenotype to obtain higher fitness. An example is associative learning where animals learn the association between stimuli and an environmental state that may affect fitness (Dukas 1998), such as the presence or the lack of food (Dukas 1998; Dukas and Bernays 2000; Egas and Sabelis 2001), or the presence of predators or competitors (Dukas 1998; Nomikou et al. 2003; Dukas 2004).

Other forms of learning may be adaptive in some ecological situations, but not in other situations. Suppose a herbivorous arthropod feeds on a certain plant resource of good quality (due to varying profiles of secondary metabolites in different plant species herbivores may be well equipped to feed on some plant species but less on other species). If it learns through sensitisation (Kandel et al. 1993; Kandel 2001) then it learns to respond to a (otherwise neutral) stimulus following an experience with another stimulus that was intense (or noxious). In this hypothetical example, the herbivorous arthropod acquires an increased responsiveness to a variety of herbivore-induced plant volatiles, after an experience with a specific volatile (or a blend of volatiles). Thus, as long as these volatiles are coupled with abundance of food the increased responsiveness acquired by such learning is adaptive, i.e., allows the animal to find more resource of better quality. If, however, conditions change such that, e.g., more conspecifics start utilizing this resource the fitness benefits from remaining on this plant resource will diminish. However, the sensitized response may lead the herbivore to remain on the same resource until the sensitized response wanes.

The effects of adaptive learning are special in that they mimic the outcomes of adaptive evolution (Papaj 1994). Adaptive learning allows individuals to modify their behaviour such that their fitness increases. Therefore, learning ability may mask genetic differences among different phenotypes and such an effect weakens natural selection and slows down the evolution of the innate trait (Falconer and Mackay 1996). An alternative hypothesis, put forward by Mark Baldwin (1896) and hence known as the Baldwin effect, holds that adaptive learning may in fact accelerate evolution of innate behaviour in novel environments, i.e., where genetically determined adaptations to the new environment have not yet evolved and the behavioural trait is under directional selection to reach a distant fitness peak. Adaptive learning not only improves the survival of
the population (thus providing the time for the evolution of a genetic basis for optimal behaviour); there is also selection for improved learning (provided heritable variation for the ability to learn the behaviour associated with higher fitness exists). If learning confers a larger fitness increase to those phenotypes (as well as underlying genotypes) that are relatively closer to the fitness peak (as postulated by Baldwin 1896) then selection for improved learning will be associated with selection for innate behaviour. According to the Baldwin effect, in a non-plastic population selection of fitter genotypes proceeds slower because there is no learning that confers additional fitness benefits to genotypes that are already closer to the fitness peak.

The Baldwin effect spurred numerous theoretical models. However, the predictions of these models did not lead to a consensus: some lent support for an accelerating effect of adaptive learning on evolution (Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; 2000: the norm of reactions model; Mayley 1997; Lande 2009), whereas others supported a decelerating effect (Andersson 1995; Ancel 2000: the quantitative genetics model; Dopazo et al. 2001). The most recent models (Paenke et al. 2007; Borenstein et al. 2006) represent an attempt at unifying these predictions and do so by defining the theoretical conditions under which one or the other effect of learning prevails. In particular, Paenke et al. (2007) argue that the curvature of the fitness landscape predicts when adaptive learning accelerates or decelerates evolution because it determines whether learning confers a larger fitness increase to those phenotypes (as well as underlying genotypes) that are relatively closer to the fitness peak (see also Egas et al. 2004 for a similar argument).

The study by Paenke et al. (2007) assumes a non-evolving learning ability – an approach that is common in the majority of theoretical studies of the Baldwin effect (Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; Mayley 1997; Andersson 1995; Ancel 2000: the quantitative genetics model, Dopazo et al. 2001; Borenstein et al. 2006). However, the emerging empirical evidence shows a genetic basis for learning ability such that it is possible to select for higher or lower levels of learning (McGuire and Hirsch 1977; Hirsch and McCauley 1977; Mery and Kawecki 2002; Dukas 2004). Hence theoretical predictions leave the question open as to how adaptive learning influences evolution of innate behaviour if it is allowed to evolve jointly with the innate behaviour. Two exceptions are the studies by Ancel (2000) and Lande (2009), where evolving phenotypic plasticity is modelled as a reaction norm (see also studies in the framework of artificial life/intelligence, e.g., Watson and Wiles 2002; Suzuki and Arita 2004). In other words, learning ability may be interpreted in these studies to be fixed at a very high level such that the most adaptive phenotype is always expressed from within the norm of reactions. The
result of these studies are consistent in that in the initial stage of evolution towards a distant fitness peak, wider norms of reaction are selected and the expression of the optimal phenotype is initially achieved through a plastic response. At the same time, the process of population movement towards the fitness peak is faster in the plastic population than in a population consisting of non-plastic individuals. However, the second stage of this process, i.e., the convergence of the population on the single optimal non-plastic phenotype (given by the fitness peak) is much slower in the plastic population. If learning has a fitness cost, then – in this second stage – it is predicted to be selected against – a process often termed as genetic assimilation in the context of the Baldwin effect (Crispo 2007; Lande 2009).

Studies by Ancel (2000) and Lande (2009) as well as earlier studies assuming non-evolving learning (e.g., Hinton and Nowlan 1987; Fontanari and Meir 1990; Ancel 1999; Mayley 1997) indicate that the rate of evolution should be measured in two stages: (1) when the population evolves towards a distant fitness peak, and (2) when the population is in the vicinity of the fitness peak, i.e., when at least some genotypes in the population express the optimal phenotype innately. Selection may favour different outcomes in these two stages and thus they may concern two different evolutionary processes.

Thus, relevant theoretical tests of the Baldwin effect should explore how various forms of adaptive learning influence evolution (1) towards a distant fitness peak and, separately, (2) in the vicinity of the fitness peak. Empirical evidence for the role of learning in evolution is virtually absent (but see Mery and Kawecki 2004) and requires a model system where genetic variation for both a behavioural trait and the ability to learn are demonstrated.

The framework of this thesis
In this thesis, I investigated whether there is genetic variation for a foraging behaviour of a predatory mite (chapters 2 and 3) and for the ability to modify it by learning (chapter 4). The behaviour in question is the response of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) to volatile compounds that are released by plants in response to plant feeding by its prey. The second part of this thesis contains a review of theoretical tests of the Baldwin effect (i.e., the hypothesis that learning accelerates the rate of evolution; chapter 5), and a theoretical study (chapter 6) wherein I investigate how the predictions of the evolution of innate behaviour change if adaptive learning is allowed to evolve.

Experimental system
The predatory mite *P. persimilis* uses olfactory cues when searching for its prey, the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) that feeds on
parenchyma cells of plant leaves (Lindquist 1998). These cues can be derived from the prey itself, such as odours emanating from faeces or silk that the prey produces abundantly on infested plants (Sabelis and Afman 1983). Another important type of cue includes volatile compounds that are released by plants upon infestation with herbivore; these volatile blends are quantitatively different from blends of uninfested plants (van den Boom et al. 2004) and they are specific to the species of the herbivore (Takabayashi and Dicke 1996). Empirical studies provide ample evidence of the attraction of natural enemies to plant volatiles induced by their prey or host in general (De Moraes et al. 1998; Turlings et al. 1990, 1995; Schnee et al. 2006; Rassman and Turlings 2007; Beyaert et al. 2009), and the attraction of _P. persimilis_ to plant volatiles induced by the spider mites in particular (Dicke and Sabelis 1988; de Boer et al. 2004a). Hence, the hypothesis was put forward that the natural enemies (parasitoids or predatory mites such as _P. persimilis_) evolved specific responses to herbivore-induced plant volatiles because such responses improve predator efficiency in prey location under natural settings (and hence fitness, although direct demonstration of fitness benefits under natural settings for _P. persimilis_ are not yet documented, and it is scarce for other model systems, see Dicke & Baldwin 2009). The hypothesis is supported by the fact that the composition of the volatile blend is specific to the herbivore species that induced it, and thus provides a signal of the presence of this herbivore. However, under natural settings there is variation in volatile blends that stems from the presence of other herbivores, from the species of the infested plants or from the odours of other uninfested plants (Schröder and Hilker 2008) as well as from abiotic conditions (Holopainen and Gershenzon 2010).

Given that _P. persimilis_ may improve its foraging success by responding to plant volatiles induced by the feeding of its prey, the presence of variation in the blends of these volatiles under natural settings raises two questions. Firstly, what features of the volatile blend trigger predator attraction, i.e., the predators may respond to specific components present in the blend, alternatively they perceive the entire blend as an entity. The damage caused to the plant by the feeding of spider mites triggers the release of a handful of novel compounds, among which methyl salicylate (MeSa) is common (de Boer et al. 2004; van den Boom et al. 2004). The emission of MeSa is not specific to the infestation by the spider mites; it is also induced by the feeding of other herbivore species (van Poeke et al. 2002; Bukovinszky et al. 2005; Zhu and Park 2005; Snoeren et al. 2010). As such, MeSa may, therefore, serve as a general signal of herbivory. Although the presence of MeSa does not always indicate the presence of spider mites, the reverse is true: feeding by spider mites almost universally triggers the emission of MeSa (for the range of plant species tested to date: Dicke et al. 1990; van den
Boom et al. 2004). Thus, using MeSa as a signal of prey presence may significantly aid *P. persimilis* in the search for prey. In line with this argument, MeSa is one of a few spider-mite induced compounds in lima bean that elicit a positive response of *P. persimilis* when offered alone (de Boer and Dicke 2004a; van Wijk et al., 2008) as well as when offered in mixtures (de Boer and Dicke 2004a). The reverse is true for many other constituents of full blends of volatiles: the presentation of a single constituent often elicits no response from the natural enemies (or herbivores) or it elicits avoidance (van Wijk et al. 2008; Webster et al. 2009). Moreover, by silencing the tomato gene encoding an enzyme crucial for the synthesis of MeSa upon herbivore infestation Ament et al. (2010) obtained the natural blend of volatiles induced by spider mites on tomato with the sole exception of MeSa. The authors subsequently demonstrated that such blend of volatiles no longer attracted *P. persimilis* as the predator did not differentiate between this blend and the blend of uninfested tomato plants. Accordingly, emerging empirical evidence for other model systems supports the hypothesis that natural enemies may respond to only a few specific compounds in the volatile blend, although this response may be dependent on the correct context of volatile presentation (such as, for example, a background of volatiles in specific ratios as in Beyaert et al. 2009; see also Schnee et al. 2006). Thus, we hypothesized that MeSa is the main feature of the blend of volatiles used by *P. persimilis* when searching for prey, although predator responses to this compound may be strengthened by providing an ecologically relevant context of other volatiles.

Secondly, the question arises as to what extent predator responses to relevant plant volatiles are genetically determined given that they can be modified by learning. Learning ability provides *P. persimilis* with one way of updating the responses to relevant compounds encountered in different contexts (i.e., with or without prey; Drukker et al. 2001; de Boer and Dicke 2004b; de Boer et al. 2005; van Wijk et al. 2008). However, predators may have also evolved genetically determined responses to those specific volatile compounds that are consistently and reliably induced by the feeding of prey, particularly if learning comes at a cost. This hypothesis is supported by evidence of genetic variation in the responses of *P. persimilis* to the volatile blends induced by feeding of the prey on the intact plant (Margolies et al. 1997; Jia et al. 2002). Furthermore, *P. persimilis* reared on spider-mite infested cucumber plants is attracted to the odours of spider-mite infested lima bean, although the blend of infested lima bean is novel in that it was never experience before (Shimoda and Dicke 2000, but see Drukker et al. 2001 for evidence to the contrary; however, in this study inexperienced predators were obtained differently: by rearing them in an odourless environment with washed eggs of the prey). The demonstrated innate preferences for the full blend of volatiles from infested plants (lima bean, in this case)
may be partly the result of the underlying innate response to a single compound shared by the blends of many plant species infested by the spider mite—a condition that is indeed fulfilled by MeSa. Van Wijk et al. (2008) found only a moderate innate attraction of *P. persimilis* to MeSa. However, their study, as well as previous studies of preference of *P. persimilis* towards single compounds or their blends (Shimoda and Dicke 2000; Drukker et al. 2001; de Boer and Dicke 2004a,b) commonly measured predator behaviour at the population level, thus in genetically variable populations.

In this thesis I tested whether genetic variation for predator response to MeSa is present in a natural population of *P. persimilis* (i.e., whether predator responses to MeSa have evolutionary potential), and to what extent any genetically fixed responses to MeSa can be modified by experience.

**Theoretical framework**

I consider phenotypes consisting of two traits: (1) an innate (i.e., genetically determined) behavioural response and (2) a learning ability. I model the innate behavioural response of a phenotype in terms of probabilities of responding to different concentrations of an environmental cue. The distribution of these probabilities is given by a Gaussian function where the mean of a Gaussian function is the evolving variable, while the variance is kept fixed and represents the exploratory range of the phenotype. In biological terms, this model of innate behaviour can be interpreted as the response of a predatory mite to a herbivore-induced plant volatile. The predator responds with certain probabilities, given by a Gaussian function, to a range of concentrations of this volatile encountered in the environment. The mean of the Gaussian function indicates the most frequently chosen concentration of the volatile.

I further assume that the innate responses of a phenotype to a range of concentrations perceived by the phenotype within its exploratory range may be modified by adaptive learning. Adaptive learning is approximated by a function that weights the phenotype’s probabilities of responding to different concentrations by the amount of fitness acquired from responding to each of these different concentrations (this is determined by a fitness function). Thus, the phenotype’s responses are adjusted such that the fitness of a phenotype is increased. However, the degree of this adaptive change of the innate responses (and the amount of fitness gain due to this change) depends on the level of learning. This level of learning is allowed to evolve jointly with the innate behaviour.

Thus, I express the phenotype as a function of two traits: the innate behaviour and the level of adaptive learning, and assume a fitness function that provides the relationship between phenotype and its fitness. This allows me to construct a fitness landscape that I use to determine the direction and the rate of
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Phenotypic evolution (Figure 1.1, an example from chapter 6); this is achieved by tracking the position of phenotype on the fitness landscape through time. The theoretical framework used in this thesis is based on the assumption that the evolution of phenotypes proceeds in the direction of increased fitness (as given by the steepest slope from any given position on the fitness landscape). Hence, starting from a given phenotype evolutionary change may proceed either via evolution of the innate response or the level of learning or via changes in both these traits. I use this framework to determine the direction and the rate of phenotypic evolution towards a distant fitness peak in two scenarios: when the initial level of learning is kept fixed and when it is allowed to evolve jointly with the innate response.

Outline of the thesis

In the first two chapters I present the results of experimental tests for genetic variation in the response of *P. persimilis* to the plant volatile MeSA using the so-called iso-female line approach (chapter 2) and a purifying selection within iso-female lines that aimed at setting apart and fixing genotypes distinct with respect to predator response to MeSA (chapter 3). Using the first approach I show that a significant amount of phenotypic variation in predator responses

Figure 1.1 – Example of a fitness landscape, i.e., the relationship between fitness (z-axis) and the bi-variate phenotype that consists of an innate response (x-axis) and a level of learning (y-axis). A black dot represents an initial phenotype and the superimposed trajectory shows the evolution of this initial phenotype towards the distant fitness peak for the innate response (i.e., towards the innate response = 0).
to MeSa is explained by variation among (genetically fixed) iso-female lines, thus providing evidence that this behaviour is genetically variable. The amount of variation explained by iso-female lines provides an estimate of the total genetic variation for predator responses to MeSa in this population and indicates that this trait has evolutionary potential, i.e., has the ability to respond to selection (Houle 1992). Moreover, I demonstrate that the addition of background volatiles of uninfested plants changes the predators’ responses to MeSa in a manner that depends on physiological state and iso-female line, thus providing evidence that these are, indeed, context-dependent.

Chapter 3 presents experiment where I selected within iso-female lines for genotypes with contrasting responses to MeSa offered in a pure compound. The selection was purifying in the sense that two groups of iso-female lines were established and in one group the lines were propagated via females that showed a preference for MeSa, whereas in the second group the lines were propagated via females that avoided MeSa. Contrary to expectations, I did not obtain two groups of iso-female lines showing preference for MeSA in the treatment group selected to prefer MeSa or avoidance of MeSa in the treatment group selected to avoid MeSa. Instead, there was a shift in the mean response to MeSa in the direction opposite to the selected one. In particular, iso-female lines selected to avoid MeSa shifted their response towards preference for MeSa while the response of the lines selected to prefer MeSa shifted towards avoidance of MeSa. Additionally, I confirm that a significant amount of variation in the responses of P. persimilis to MeSa is explained by iso-female lines.

These first two chapters consistently document that there is a significant amount of variation in predator responses to MeSa due to iso-female lines, thus supporting the hypothesis that this behaviour is genetically variable. Future studies will address whether the revealed genetic effects are additive (and thus predict the response to directional selection) or non-additive.

Chapter 4 provides evidence that the genetically determined responses of P. persimilis to MeSa can be modified by experience with this compound. This is inferred from a change in the average responses of iso-female lines detected after they were fed or starved in the presence of MeSa. The change in behaviour represented either increased or decreased responsiveness to MeSa in a way that was independent of the nutritional context of this experience (i.e., whether MeSa is experienced in the presence or absence of food), thus suggesting that learned responses of P. persimilis are shaped by the amount of MeSa they were exposed to and thus may be based on a non-associative mechanism. Moreover, I found variation among the iso-female lines in the way they responded to the experience, and this result provides support for the hypothesis that the predator’s ability to modify responses to MeSa also has a genetic component.
In the final two chapters I review and expand the theory of the Baldwin effect, i.e., on the hypothesis that adaptive learning accelerates the rate of evolution of innate behaviour. In chapter 5, I review theoretical studies of the Baldwin effect that provide contrasting predictions of either accelerating or decelerating effects of learning on evolution. I discuss the dependence of the predictions of these studies on the critical assumptions such as non-evolving learning ability and the effect of learning on phenotype’s fitness.

The question of how relaxing the assumption of non-evolving learning changes evolutionary predictions is dealt with in chapter 6. Therein, I model the evolution of an innate behavioural response of a phenotype to an environmental cue where this behaviour can be modified by costly adaptive learning within a preset exploratory range of cue-values. Evolutionary predictions are compared under two scenarios: (1) when innate behaviour evolves towards a distant fitness peak while the learning ability is kept fixed, and (2) when learning ability is allowed to evolve jointly with the innate behaviour. This comparison reveals that allowing adaptive learning to evolve greatly reduces the time to reach the fitness peak when (1) the net effect of learning on phenotypic fitness is large (i.e., learning modifies innate behaviour within a large exploratory range of cue-values), (2) learning has a fitness cost, and (3) the initial level of learning is higher than the evolutionary optimum. Under these conditions there is a critical threshold level of learning above which learning is favoured by selection and evolves towards an optimum level and below which it is selected against. Thus, I find a threshold level of learning above which joint evolution of learning and innate behaviour is accelerated. When the net effect of learning on phenotypic fitness is small (i.e., learning modifies innate behaviour within a small exploratory range of cue-values) evolution of the phenotype proceeds effectively only via changes in the innate behaviour. Together these results allow me to conclude that assuming a form of fixed learning is only justified when the net effect of learning on phenotypic fitness is small.

References
Introduction


Chapter 1


